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Bean Pod Mottle Virus and Cowpea Mosaic Virus in Louisiana Soybeans.

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Louisiana State University and Agricultural & Mechanical College

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BEAN POD MOTTLE VIRUS AND COWPEA MOSAIC VIRUS IN LOUISIANA SOYBEANS.

The Louisiana State University and Agricultural and Mechanical College, Ph.D., 1971
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BEAN POD MOTTLE VIRUS AND COWPEA MOSAIC VIRUS IN LOUISIANA SOYBEANS

A Dissertation

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy

in

The Department of Plant Pathology

by
Kenneth Cosmas Gremillion
B.S., Louisiana State University, 1965
M.S., Northeast Louisiana University, 1968
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ABSTRACT

Bean pod mottle virus (BPMV) and cowpea mosaic virus (CPMV) were found in soybeans (Glycine max Merr.) and in cowpeas (Vigna sinensis Torner), respectively in Louisiana. Both viruses were identified by insect transmission, physical properties, serology, host range and symptom expression.

BPMV produced a mottling on the leaves of soybean, while CPMV caused a severe mosaic and stunting of soybean plants. Both viruses had a wide leguminous host range, and BPMV was found to overwinter in Desmodium paniculatum L. The optimum temperatures for symptom expression were between 20-30 C. Symptoms were supressed at 35 C and none appeared at 40 C.

The physical properties of BPMV and CPMV were very similar, both with a dilution end-point of 10^{-5}, a thermal inactivation point of 60-70 C and the longevity in vitro between four and six days. The North Carolina isolate of BPMV was related to the Louisiana isolate by the Ouchterlony gel-diffusion test. CPMV antigen formed precipitn zones with the California antiserum, indicating that the viruses were related. The electronmicroscopy of BPMV and CPMV showed both to be isometric particles of approximately 30 Mu in diameter.

Field tests revealed no significant differences in yields between the inoculated BPMV plants and the controls. However, in greenhouse tests there were significances in the yields of BPMV infected Hill, Dare and Lee soybeans and the controls. No differences were found between treated and healthy Davis, Semmes and Bragg. There

were significances between CPMV infected plants and controls both in the field and the greenhouse. Hill was the only variety in the field in which the yields of CPMV inoculated plants were not significantly lower than the controls. In the greenhouse the yields of Hill were severely reduced by the virus.

Ceratoma trifurcata Forst. (bean leaf beetle) was found to be a vector for both BPMV and CPMV. Myzus persicae Sulz. (green peach aphid) failed to transmit either virus. Neither virus was seed transmitted.

INTRODUCTION

Soybeans Glycine max Merr. have steadily increased in importance as a commercial crop in the United States (57). Because of their high protein content, soybeans are used extensively in animal feeds. Soybean oil is used in the manufacture of plastics. Presently, their value as food substitutes is becoming increasingly important.

In 1924, 1782 acres of soybeans wer planted in the United States. By 1970, this figure had risen to 41.5 million acres with a total production of 1,134 million bushels. Soybean production in Louisiana has increased at the same pace until it ranks as the number one crop in acreage. Over 1.5 million acres were planted in soybeans in Louisiana in 1970 which yielded 38.5 million bushels. The average yield is about 25 bushels per acre for the state.

Any soybean disease reduce yields. The degree of loss depends on the disease, the severity of the disease, and the percentage of plants affected. Annual losses in the United States from all soybean diseases have been estimated at 12 percent of the total crop (14, 20). Although soybean virus diseases in Louisiana are not yet of major importance, the potential for development of the disease exists. However, a five acre planting at Bayhill, Louisiana was observed to ba a total loss because of infection with BPMV and tobacco ringspot virus (TRSV). Other isolated plantings in West Baton Rouge and Iberville Parishes were observed to be nearly 100 percent infected with BPMV or soybean mosaic virus (SMV). Fields of lesser percent infection were observed in scattered locations of Louisiana (4, 29).

The two soybean viruses investigated in this study were BPMV and cowpea mosaic virus (CPMV). Both viruses belong to a group of spherical viruses of which CPMV is the type member (17, 33). They possess a rather wide leguminous host range and some non-leguminous species. BPMV produces a striking systemic mottle on soybean trifoliate leaves, and some vein-clearing and chlorosis. CPMV infected soybean plants are severely stunted, with a coarse mosaic pattern. Vein banding and vein-clearing are also evident. Both viruses are transmitted by the bean leaf beetle (Ceratoma trifurcata Forst.) which is the most common vector of the viruses (29). Both are easily transmitted mechanically.

The large number of viruses capable of infecting soybeans (32, 39, 69) coupled with increased soybean production in Louisiana enhances the probability of multiple virus infections. Walters (61) reported SMV, BPMV, yellow stipple virus (YSV), and an unidentified virus occurring naturally in the Arkansas river valley. Ross (44) found SMV and BPMV coinfecting soybeans in North Carolina and Virginia.

This dissertation was designed to identify the two isolates of BPMV and CPMV and to verify by symptomatology, host range, physical properties, insect transmission and serology that they were indeed those isolates described in the literature, or closely related to them. A second objective was to determine the reaction of six Louisiana soybean varieties to BPMV and CPMV and their interation with the viruses. Field and greenhouse yield tests were conducted to determine if yield losses did occur due to the viruses, and if so, the degree of loss and significance of infection.

LITERATURE REVIEW

Bean pod mottle virus (BPMV) was described by Zaumeyer and Thomas (68) in 1948, as a new virus on the basis of host range and physical properties. The virus derives its name from the severe mottle on the pods of Phaseolus vulgaris L. var. Tendergreen. Zaumeyer and Thomas (68) compared BPMV to southern bean mosaic virus (SBMV), which it most closely resembles. It was not until 1958, however, that Walters (61) reported the virus present in soybean fields in Arkansas.

The first report of CPMV was made by Elliot (21) in 1921, in Arkansas. By 1929, the disease was reported from Oklahoma, Louisiana, Indiana, Georgia, Iowa, Mississippi, Kansas, and New Jersey (34). McLean (34) in 1941, described a mosaic disease of cowpeas (Vigna sinensis Torner) var. New Era and Whipoorwill that caused severe dwarfing of the plants in Oklahoma. He later identified the virus as CPMV on the basis of varietal susceptibility, host range, physical properties and methods of transmission. Similar virus diseases of cowpea have been reported in China (67), Nigeria (12), and Trinidad (12, 13, 19). Because of slight differences in their descriptions it was suggested that three strains of CPMV existed (50). Shepherd and Fulton (50) described three mosaic diseases of cowpea or its close relatives. One type was aphid-transmitted, had a low dilution end point, a brief longevity in vitro and its host range was limited to the Leguminosae. A second type was similar, except that it had a wide non-leguminous host range. The third type characteristically had a

higher dilution end point, a longer period of aging in vitro, was transmitted by chewing insects and its host range was limited to the Leguminosae.

Symptomatology of any virus on the basis of macroscopic symptoms alone has been shown to be of limited usefulness, especially when used to differentiate strains (1, 6). Bawden (7) was of the opinion that variability, especially in symptomatology and host range, was normal rather than exceptional. Environmental factors and the symptoms produced varied and unless the environment was defined, conditions could have been described which may never again be precisely reproduced. Zaumeyer and Thomas (68) found that BPMV produced local lesions on some varieties of beans and systemic mottle on others. Those varieties susceptible to local infection were immune from systemic infection, and those susceptible to systemic infection were immune from local infection. The circular local lesions appeared three to four days after inoculation and the edges of the lesions were located near the veins, the vascular tissue became necrotic. Zaumeyer and Thomas (68) reported that BPMV produced a severe mottle on P. vulgaris var. Longreen and Tendergreen. The trifoliate leaves of Full Measure and Tendergreen were both mottled and necrotic, and death of the plant occurred in some cases. The pods of many susceptible varieties were severely mottled and darker green than normal. They were shorter than normal, malformed, curled, twisted, and somewhat rough and warty; often they contained abortive or abnormally developed ovules. Ross (43) described the synergistic action of BPMV and soybean mosaic virus (SMV) on Hill and Lee soybeans. The apices of the plants

developed a shock reaction i.e., curvature and death of the shoot terminal, when primary leaves were inoculated.

McLean (34) described CPMV symptoms that developed from seed of CPMV infected plants. Primary symptoms of mosaic appeared in the simple leaves. Vein-clearing was the most noticeable symptom in the developing leaves. Following this, a mottling of light and dark green was evident. There was little difference in the characteristic symptoms produced in the first compound leaves or the succeeding ones in plants infected from seed or those secondarily infected. Irregular patches of a lighter green were found interspersed with the normal green. These patches were often elongated blotches running parallel with the veins of the leaflet. Accompanying the mottling there was frequently a convex cupping or arching of the leaflets. McLean (34) also found that plants grown from infected seed exhibited shortened internodes, and the petioles were frequently twisted and shorter than those on healthy plants. The plants were late in maturing due to the systemic infection. There seems to be no method of predicting where susceptible plants may be found for unknown viruses. Certain families, Leguminoseae, Cucurbitaceae, Solanaceae, and Chenopodiaceae contain species that are susceptible to a wide range of viruses (24). Zaumeyer and Thomas (68) used practically all the commercially important varieties of snap and dry beans in a host range study with BPMV. Of the 68 varieties or strains inoculated with BPMV, 41 produced local lesions, 23 were susceptible to systemic lesions, and 4 had both local lesions and systemic infection. Zaumeyer and Thomas (68) found that of these 68 varieties, the majority of the green-podded varieties of snap and dry beans were susceptible to local infection. Most of the

varieties of lima beans were inoculated with BPMV. Two small-seeded varieties, Green-seeded Henderson Bush, and U. S. 343, were susceptible to local lesion infection only. Fordhook, U. S. 242 and Early Market, all large-seeded varieties, were immune. In addition to the snap and dry bean varieties, Zaumeyer and Thomas (68) inoculated 25 species representing 20 genera in nine families. None of these species became infected except soybean which showed systemic infection. The following varieties of soybeans were susceptible: Arksoy, Biloxi, Burdette, Cherokee, Chief, CNS, Gibson, Illini, Lincoln, Rolsoy, and Virginia. Walters (61) also reported BPMV as infecting soybean fields in Arkansas. Desmodium paniculatum (beggarweed), a wild legume showing virus symptoms, was collected by Walters (61) and Moore et al. (35) in the area of the infected soybean fields. Walters (61) inoculated various plants with extracts from the infected D. paniculatum leaves. Those plants showing systemic symptoms were Hill and Hood soybeans (G. max), "Black Valentine" bean (P. vulgaris), and Monarch cowpea. Pinto, Great Northern, and Bountiful beans showed local infection. No infection occurred on cucumber (cucumis sativus L.), Nicotiana tabacum L., N. rustica L., or N. glutinosa L. Serological tests showed that the virus from D. paniculatum was serologically identical to BMPV. In a similar study, Horn et al. (29) reported BPMV present in soybean fields in Louisiana. They also found D. paniculatum and D. canadense (61) to be hosts for BPMV.

Skotland (52) found BPMV in naturally infected soybeans in North Carolina and Virginia. All soybean varieties included in the test were susceptible. The virus produced local lesions or mottling on various bean varieties. Some varieties of cowpeas were resistant

and others were susceptible to infection. Annual and perennial Lespedeza spp. (lespedezas), Stizolobium deeringianum L. (velvet bean) and Trifolium incarnatum L. (crimson clover) were reported as hosts for the first time (51). Phaseolus lunatus L. (lima bean), Pisum sativum L. (garden pea), Melilotus officinalis (L.) Lam. (sweet clover), Crotalaria spp. (rattlebox), Vicia faba L. (broadbean), Arachis hypogaea L. (peanut), Medicago sativum L. (alfalfa), Nicotiana tabacum L. (tabacco), Cucumis sativus L. (cucumber), Capsicum annum L. (pepper), Lycopersicon esculentum Mill. (tomato), and Petunia spp. (petunia) were resistant (52).

Gardner (25) found that a number of varieties of cowpea (V. sinensis) var. Progressive White in Indiana were susceptible to CPMV. McLean (34) reported that 13 of 19 varieties of cowpeas tested were susceptible to CPMV. The lima bean (Phaseolus lunatus macrocarpus Benth. var. Wood's Prolific) was the only other member of the Leguminosae susceptible to CPMV (34).

Shepherd and Fulton (50) described a virus that induced a mosaic disease in Early Wilt Resistant Ramshorn cowpea (V. sinensis) and whose host range was restricted largely to the Leguminosae. This isolate was closely related serologically to southern bean mosaic virus (SBMV), although it was not infectious to seven varieties of beans tested. They reported a rather limited host range for their CPMV isolate. Cyamopsis tetragonoloba (L.) Taub. showed local lesions in inoculated leaves and very faint systemic vein-clearing or mottle. G. max var. "Hawkeye" showed a systemic chlorotic mottle. Pisum sativum L. var. Little Marvel and Vigna sesquipedalis (L.) Fruwirth were both systemically infected. The workers were unable

to induce virus infection to members of Chenopodiaceae, Cucurbitaceae, and Solanaceae. Other common members of the Leguminoaae were immune despite repeated inoculations. In a later study, Shepherd (51) reported a CPMV isolate that had a broader host range than those previously reported and which was mechanically transmissable to several non-leguminous plants. Non-leguminous plants reported by Shepherd (51) as producing a reaction to CPMV were: C. sativus L., Nicotiana spp., Datura stramonium L., Petunia hydrida Vilm., Beta vulgaris L., Gomphrena globosa L. Chenopodium amaranticolor L., and C. capitatum (L.) Asch. The following species of Leguminosae were sesceptible: Cassia occidentalis L., Cajanus cajan (L.) Millsp., C. tetragonoloba (L.) Taub., Dolichos lablab L., G. max (L.) Merr., Phaseolus aureus Roxb., P. coccineus L., P. lunatus L., P. sativum L., Sesbania exalta (Raf.) Cary, and V. Sesquipedalis.

Chant (13) described the host range of the Trinidad strain of CPMV. On cowpea (Vigna unguiculata) (L.) Walp. chlorotic lesions appeared on the inoculated leaves, followed by pronounced vein-clearing on the first trifoliate with subsequent leaves showing regular yellowish and dark green mottling with some blistering and distortion of the lamina. Local lesions were produced on Canavalia ensiformis (Sword Bean), Mucuna aterrima Holland (Bengal Bean), P. vulgaris var. Comtesse de Chambord, C. amaranticolor, and P. hybrida.

Many viruses were reported to infect soybean (30, 31, 32) but few reports indicated the effects of viruses on soybean yields. SMV caused a reduction of yields of soybeans in Indiana by 30-75 percent in 1921 and 1922 (25). More recently, Crittenden, et al. (18) obtained soybean losses of approximately 60 percent when 85 percent of the

plants were infected with tobacco ringspot virus (TRSV). The large number of viruses capable of infecting soybeans (18, 39) coupled with expanding soybean production, increases the probability of multiple virus infections. Walters (61) reported SMV, BPMV, yellow stipple virus, and an unknown virus occurred naturally in soybeans in the Arkansas River Valley. Losses in most fields were minor, but a total loss occurred in some fields. Skotland (52) found up to 75 percent infection by BPMV in naturally infected soybeans in North Carolina and Virginia. Ross (41, 44) showed that early combined infections with SMV and BPMV on Hill and Lee soybeans drastically reduced soybean yields and reduced the crop value by adversely affecting seed characters. Plants of both varieties were slightly stunted by BPMV. the doubly infected plants developed chlorotic mottling, stunting, curvature and brittleness of stem apices, and dark streaks on filled pods. Yield reductions of Hill inoculated with either strain of SMV in combination with BPMV were significantly greater than the sum of the reductions caused by separate inoculations with SMV or BPMV. Yield response of Lee infected with SMV and BPMV was synergistic (80 percent reduction), but the yield reduction of Lee infected with both viruses was not synergistic (65 percent reduction). BPMV caused greater yield reduction to Lee than to Hill. In a later study, Ross (45) stated that sequential inoculations with SMV and BPMV (one week apart) in either order reduced yields as much as simultaneous inoculations. Sequential inoculations of SMV followed by BPMV caused more seed mottling, greater reductions in seed size, and poorer seed quality than inoculations made in the reverse order.

Comparatively little work has been done concerning varietal response of soybeans or cowpeas to CPMV. However, Chester (15) estimated the loss of cowpeas at 30 percent in several fields in Oklahoma in 1939 and stated that the disease was seen frequently at various locations in the state.

The influence of temperature on the expression of symptoms of BPMV was first described by Zaumeyer and Thomas (68). The optimum temperature for production of local lesions was 10 C. Bancroft (6) and Ross (44) also reported variation in symptom expression of BPMV which they attributed to increase in temperature. BPMV systemic symptoms faded or were masked as temperatures increased.

BPMV was transmitted by the bean leaf beetle (C. trifurcata) (41, 62), the most common vector of the virus. Walters (62) reported 53 percent transmission to Hill soybeans with bean leaf beetles when he allowed the insects a 48 hours acquisition period on virus infected D. paniculatum plants. Ross (42) found bean leaf beetles naturally infecting soybeans with BPMV in North Carolina. In greenhouse tests he was able to obtain 67 percent transmission of BPMV by the beetles. Horn et al. (29) reported that in Louisiana the chrysomelid beetle vectors of BPMV readily feed on Desmodium spp., a natural host for BPMV, and then transmit the virus to soybeans early in the growing season. Horn et al. (29) conducted greenhouse tests with the bean leaf beetle and other chrysomelid beetles to determine if transmission of BPMV occurred. Transmission of BPMV by these insects ranged from 4-15 percent.

The first report of insect transmission by insects of CPMV was in 1924 by Smith (53). Since then others have reported transmission

of CPMV by the bean leaf beetle (Ceratoma trifurcata Forst.). In Trinidad, Dale (19) described a mosaic disease of cowpea that was transmitted by bean leaf beetles (C. ruficornis Oliv). Anderson (2) and Yu (67) have reported aphid-borne viruses restricted to the Leguminosae in host range and having similar properties in vitro to the Trinidad virus.

Seed transmission of CPMV has been reported by Anderson (2, 3), Dale (19), McLean (34), Shepherd and Fulton (50), Yu (66), Snyder (54), and Gardner (26). Most of these recorded 10 percent seed transmission, however, McLean (34) reported 2-6 percent transmission and Shepherd and Fulton (50) 3-4 percent transmission. No seed transmission of BPMV has been reported.

A number of workers have investigated the physical properties of BPMV (6, 33, 68) and CPMV, respectively (12, 13, 34, 47, 48, 49, 58, 59, 67). However, the data presented was inconsistent. Zaumeyer and Thomas (68) reported thermal inactivation points for BPMV to be between 70 and 75 C when heated for 10 minutes. This agrees closely with Matthews (33) who listed the thermal inactivation points between 60 and 85 C for that group of spherical viruses of which CPMV and BPMV are members. McLean (34) obtained infectivity with CPMV extract up to 72 C, but not with extract heated up to 75 C. Yu (67) reported a thermal inactivation point of 64 C for CPMV. Shepherd and Fulton (50), however, got infection at 85 C, but none at 90 C with heated CPMV extract. Shepherd (51) later reported a much lower thermal inactivation point for CPMV. He observed infection at 65 C but none at 70 C. Chant (13) also reported inactivation of CPMV at 70 C.

A wide range of values has also been reported for the dilution end point of BPMV and CPMV, respectively. The maximum dilution at which infection by BPMV occurred was reported by Zaumeyer and Thomas (68) to be 1:10,000. McLean (34) found that the dilution end point for CPMV was 1:1500 while Yu (67) observed no infection at 1:2560. Shepherd and Fulton (50) and Shepherd (51) and Chant (13) determined the dilution end point of CPMV was 1:10,000.

Longevity of BPMV in vitro has been determined to be 62 days at 18 C (68). Chant (13) stored CPMV extract at 18 C and noted infection at eight days but none at 16 days. McLean (34) aged CPMV extract at 20-25 C and reported inactivation of the virus between 48 and 72 hours. Yu (67), however, got infection at 72 hours but none at 96 hours. Shepherd and Fulton (50) reported infection with CPMV extract stored at 22 C after 15 days but none at 20 days, and Shepherd (51) at 7 days, none at 10 days.

Morphologically, BPMV and CPMV are very similar. Matthews (35) listed them in the Squash Mosaic Virus Group. This group consists of small isodiametric viruses which are more or less distantly related serologically, and transmitted by beetles. Included in the group are squash mosaic virus, BPMV, and CPMV, all of which produce several sedimenting components (66).

BPMV has been shown to be composed fo three components, each composed of polyhedral particles of a diameter of about 30 Mu (6, 23, 27, 47). In purified preparations of BPMV the three types of particles are separable by their sedimentation characteristics (11). The top component (54S), middle (91S) and bottom (112S) contained 0, 30 and 37 percent RNA, respectively (6). The isolated RNA from the middle

component was not infectious, and had a relatively lower content of guanylic and uridylic acids compared with the bottom component (47). When components were isolated, and tested for infectivity, either singly or in mixtures, the infectivity of the bottom component was increased by the middle component but not by the top component. This fact was attributed to RNA, the content of the middle component (65).

Shepherd (51) demonstrated CPMV to be three spherical particles of 30, 29 and 30 Mu, respectively. Van Kammen (58) found that purified preparations of CPMV contained three components separable on centrifugation with 59S, 95S, and 115S and estimated RNA content for top, middle, and bottom components as 0, 23, and 32 percent RNA, respectively. These figures showed the remarkable similarity of the two viruses. The base composition of the middle RNA of CPMV was richer in uridylic acid than that of bottom RNA. Van Kammen (59) showed that neither component alone was infectious, but when the two were mixed, infectivity was restored.

Serology has proven useful in establishing relationships of many plant viruses (5, 46). Bennet (8) was of the opinion that serology was more important than differential hosts and physical properties in differentiating strains of most plant viruses. Scott et al. (46), using the microprecipitin test, pointed out that the suspected relationship between BPMV and red node virus did not exist.

The Ouchterlony agar double diffusion test represents one of the newest serological techniques (5). Scott et al. (46) showed that BPMV and red node viruses were serologically unrelated, each giving distinct lines of precipitation. Shepherd (51) showed that the Arkansas and Trinidad strains of CPMV were closely related, but not

identical serologically. Both viruses produced strong precipitation lines when tested against either the homologous or heterologous antiserum. Both viruses reacted with antiserum to BPMV. In reciprocal tests, BPMV produced diffuse, but definite, precipitin bands when tested with antisera to either of the cowpea viruses. The Nigerian cowpea yellow mosaic virus (CYMV) consistently reacted with each of the cowpea viruses, but did not react with BPMV. This suggested a distant relationship between the Nigerian CYMV and BPMV (51). Shepherd demonstrated a similar relationship between the Arkansas and Trinidad cowpea viruses and BPMV. Shepherd (49) was also able to show that a CPMV strain, recovered from cowpea seedlings, was closely related serologically to southern bean mosaic virus (SBMV). Walters (63) confirmed the presence of BPMV is D. paniculatum when the crude extract of the infected plants reacted with BPMV antiserum in the Ouchterlony gel-diffusion test.

A rapid identification of viruses can be obtained for reasonable concentrated preparations by mixing the virus solution with a stain. Semancik and Bancroft (47) described a method for electron microscopy of BPMV particles. Fractionated preparations were stained in a 1:2 mixture of virus suspension to 2 percent uranyl acetate. After a 3-6 hour staining period, carbon-coated Formvar grids were coated with the virus-stain mixture. Shadowed preparations revealed spherical particles of about 30 Mu in diameter.

Shepherd (51) took measurements from electron micrographs of purified preparations of the Arkansas and Trinidad viruses and CPMV negatively stained with phosphotungstate showed uniform spherical particles calculated to be 30, 29, and 30 Mu diameter, respectively.

Similar preparations of the Arkansas virus shadowed with uranium indicated the particles were polyhedral with a mean diameter of approximately 33 Mu. Chant (13) stained the Trinidad CPMV strain with phosphotungstate and observed polyhedral particles with a diameter of approximately 25 Mu.

MATERIALS AND METHODS

Source of Isolates

The BPMV isolate used in this study was collected during an examination of soybean fields in Louisiana in 1968. The CPMV isolate was obtained from infected cowpea plants on the Louisiana State University Hill Farm. Both isolates were maintained in Dare soybeans in the greenhouse.

Method of Inoculation

Mechanical inoculations, unless otherwise stated, were made with freshly expressed sap mixed with an equal volume of 0.01 M phosphate buffer pH 7.0. The inoculum was applied to the bifoliate leaves or cotyledons by rubbing between the thumb and forefinger. In the case of local lesion hosts and cowpea plants which have larger leaves, a gauze pad was used to apply the inoculum. Carborumdum, 600 mesh, silicon carbide, was used as an abrasive (40).

Virus Assay

All assays of BPMV and CPMV were done on one of two local lesion hosts, *P. vulgaris* var. Pinto or Great Northern.

Host Range Studies

The following groups of plants were mechanically inoculated with BPMV and CPMV, respectively: 1) wild legumes grown from seeds collected in Louisiana, 2) cultivated legumes grown from commercial seed and 3) cultivated vegetables grown from commercial seed. In

addition, Desmodium spp. collected in Louisiana and naturally infected were assayed for virus on Pinto and Dare soybean indicator plants. All seeds were germinated in flats and inoculations were made on the first true leaves (bifoliates) or cotyledons of non-leguminous species. One row of 12 seedlings in a flat comprised one treatment. Each treatment was replicated four times. Twelve seedlings per flat not rubbed with virus were the control plants. The tests were conducted in an air-conditioned greenhouse at 27 C.

Environmental Effects

To determine the effect of temperature on the symptom expression of BPMV and CPMV, Dare soybean plants were inoculated with each virus, respectively, and placed in environmental growth chambers (Scherer Mobile Greehnouse) at 20, 25, 30, 35, and 40 C. Each treatment was replicated four times. Every chamber received 14 hours of light at the specified temperatures and 10 hours of darkness all at 21 C to proximate night temperatures. The plants were observed daily and symptoms recorded.

Field Testing

Six soybean varieties were used to test the effect of BPMV and CPMV on the yields of beans in 1969 and 1970. Hill, Dare, Lee, Bragg, Semmes, and Davis seeds were obtained from the Alexandria Seed Co., Alexandria, Louisiana. The tests were placed on the Louisiana State University Hill Farm in a superimposed factorial arrangement on a randomized block design. All plots consisted of four rows and only the two center tows were treated. Four buffer rows separated each plot. The plots were 50 feet long, with 25 foot subplots. A 10 foot

alley was cut between each subplot. Each treatment was replicated four times.

The seeds were planted with a Planter IV Junior at the rate of 20 seeds per foot. Inoculations were made when the bilateral leaves were well expanded and before the first trifoliates budded out. Dare bean leaves from BPMV and CPMV infected plants, reared as inoculum plants in the greenhouse were minced in a Waring Blendor in 0.01 M phosphate buffer. Carborundum was added as an abrasive. The two bifoliate leaves were inoculated in the manner previously described. The plants were harvested when the beans were sufficiently dried and shattered with a plot thrasher and the weights of the beans recorded.

Greenhouse Yield Tests

The effect of BPMV and CPMV, respectively, on the same six varieties were tested in the greenhouse. The plants were grown in 14 inch pots with seven plants per pot. The pots were randomized on the greenhouse bench and each treatment was replicated four times. The plants were inoculated as described previously. Appropriate controls were used. At maturity, the beans were harvested and air dried at room temperatures and humidity. After sufficient drying, the pods were shelled by hand and the weight of beans from individual pots were recorded.

Transmission Studies

A) Insect Transmission

C. trifurcata (bean leaf beetles) were used as vectors for BPMV, and the Myzus persicae Salz. (green peach aphid) for CPMV. Bean leaf beetles were starved for 4-5 hours then allowed to feed on infected leaves in small vials. At the end of the feeding period, individual

beetles were transferred from the vials to Dare plants in cages. The cages were constructed of sheet cellulose nitrate rolled into a cylinder, sealed and capped at one end with netting. Sponge rubber stoppers in the sides of the cages could be removed from the cages to serve as an opening to place the insects inside. The open ends of the cages were placed over the plants and pushed into the soil. The beetles were allowed to feed until they were observed to feed before they were transferred to the healthy plants. Green peach aphids were reared on young Datura spp. plants and transferred every 12-14 days to fresh host plants. Aphids were brushed into glass petri dishes and starved for 30 minutes to one hour before the acquisition period. A dissecting microscope was used to observe and verify that the aphids were feeding or at least probing. Then they were transferred to the test plants where they were allowed to remain for eight hours. Observations were made daily for foliar symptoms. In case the leaf symptoms of any individual plant was questionable, expressed sap from that particular plant was used to assay the virus on Pinto beans. Aphids which were fed on healthy leaves served as the controls. Twenty plants were used for each treatment.

B) Seed-borne Transmissions Tests

Seeds from Hill, Lee, Dare, Bragg, Semmes and Davis plants that had been inoculated in the trifoliate leaf stage with BPMV and CPMV, respectively, were germinated in the greenhouse during February and March at which time temperatures ranged from 21 C to 32 C. The seedlings were grown to the second trifoliate stage. The number of

seedlings showing symptoms were recorded and the percent infection calculated on the basis of total germination.

Physical Properties

A) Dilution End-point (DEP)

One ml of undiluted, infective BPMV and CPMV sap, respectively, was put into a test tube with 9 ml of distilled water. Nine ml of distilled water were poured into six more test tubes. One ml of the sap was pipetted into one tube containing 9 ml water. From this diluted sap one ml was put into another test tube with 9 ml of water. The procedure was repeated until sap dilutions of 10^0 - 10^{-6} were made. The dilutions were assayed on Great Northern beans for infectivity. DEP was reported at the dilution at which no local lesions were produced on Great Northern. Twelve plants were used to assay each dilution.

B) Thermal Inactivation Point (TIP)

BPMV and CPMV infective plant sap, respectively, were put into capillary tubes. One end of the tube was sealed by holding over a flame, and the other end was inserted into a slit in a cork. Four tubes of both viruses, respectively, were prepared for and tested at 60, 70, 80, and 90 C by floating them in an agitated water bath for ten minutes. Immediately after treatment they were placed in ice water where they remained until thoroughly cooled. The contents of each tube was assayed on local lesion hosts.

C) Longevity in Vitro (LIV)

Infective sap of BPMV and CPMV, respectively, was sealed in small stoppered bottles at 20-22 C. Beginning two days after storage, local lesion indicator plants were inoculated with the sap and incubated in the greenhouse.

Purification

A) Purification of BPMV

A modification of Bancroft's (6) method with chloroform-butanol for the purification of BPMV from infected soybean plants was used. About 500-700 of soybean leaves that had been previously frozen, were thawed and macerated in a meat grinder to which was added 3.5 ml of 50 percent K_2HPO_4 per 100 grams tissue. The juice was squeezed through two thicknesses of cheesecloth and the remaining fiber was extracted with 150-200 ml of 0.01 M pH 7.0 phosphate buffer. Both extracts were combined and mixed with gentle stirring with equal volumes of n-butanol and chloroform (55). The emulsion was broken by low speed centrifugation and the supernatant solution was stored overnight at 4 C. Clarification was completed by centrifugation at 10,000 rpm for 10 minutes in a Sorvall RC-2 refrigerated centrifuge. The supernatant solution was then centrifuged for two hours at 28,000 rpm in the no. 30 rotor of a Spinco Model L preparative ultra-centrifuge. The pellets were resuspended in 0.01 M pH 7.0 phosphate buffer, clarified by low speed centrifugation and centrifuged at 38,000 rpm for 1.5 hours in the no. 40 rotor using the Spinco Model L ultracentrifuge. The pellets were suspended in 5-6 ml of 0.5 M pH 7.0 phosphate buffer, dialyzed for 24 hours at 4 C against that buffer,

clarified and resedimented in the no. 40 rotor. The pellets at this stage were colorless and refracted light so as to appear crystalline. The final pellet was suspended in 0.2 M pH 7.0 phosphate buffer and frozen.

B) Purification of CPMV

The purification of CPMV was accomplished by the polyethylene glycol method (28) described by Van Kammen (58). The primary leaves of cowpea seedlings were inoculated when fully expanded, just before the secondary leaves began to appear. When the symptoms appeared on the trifoliates, the bifoliate and trifoliate leaves were harvested and frozen until used for purification of the virus.

Frozen infected leaves were homogenized with an equal amount of 0.1 M phosphate buffer pH 7.0 in a Waring Blendor. The homogenate was pressed through cheesecloth and the extract was clarified by centrifugation at 10,000 rpm for 15 minutes. Polyethelene glycol 6000 (PEG) was added to produce a final concentration of 4 percent (w/v) and enough salt to make it a 0.2 M NaCl solution was added. The mixture was stirred at room temperatures to dissolve the PEG and NaCl. After one hour the precipitate was collected by centrifugation at 10,000 rpm for 15 minutes and the pellets were resuspended in 0.01 M phosphate buffer pH 7.0. This suspension was clarified by centrifugation at 10,000 rpm for 15 minutes. It was further purified by two cycles of differential centrifugation at 30,000 rpm in the no. 30 rotor of the Spinco Model L preparative ultracentrifuge for 3 hours to sediment the virus and at 10,000 rpm for 15 minutes in 0.01 M

phosphate buffer pH 7.0 to clarify the virus suspension. Further purification was obtained by differential centrifugation as described above.

Serology

A) Production of Antisera

Antiserum to BPMV and CPMV was prepared by intramuscular infections (6, 16) of partially purified virus into a rabbit previously bled for normal serum. The antigen for intramuscular injection was prepared by mixing with an equal volume of Freund's complete adjuvant. This mixture was thoroughly agitated for 10-15 minutes in a Sorval Omni-Mixer. One ml of the mixture was injected into each hip of the rabbit. Antigen prepared for footpad injections was prepared in the same manner except that Freund's incomplete adjuvant was used. One-quarter ml of this mixture was injected interdermally in each footpad. At the end of a 3-week period serum was obtained. All serum was mixed with an equal volume of glycerol then frozen.

B) Agar Diffusion Tests

The Ouchterlony (36, 37) agar gel diffusion method was used to test the reactions of BPMV and CPMV, respectively, to several antisera including that produced in this study. Antisera to BPMV were obtained from the following sources: Dr. J. B. Bancroft, Purdue University, Dr. J. P. Ross, North Carolina Aricultural Experiment Station, and Dr. H. J. Walters, University of Arkansas. Dr. J. P. Ross also supplied BPMV antigen. Antiserum to CPMV was obtained from Dr. R. J. Shepherd, University of California, Davis

Twelve and one-half ml of 0.85 Ion Agar No. 2 (Consolidated Laboratories, Chicago Heights, Ill.) containing 0.4 percent sodium azide were poured into each standard nine cm plastic petri dishes. Five well patterns were used in this test. One pattern had a center well and eight peripheral wells seven mm from the edge of the center well. Another pattern consisted of a center well surrounded by four equally spaced peripheral wells of the same size and spacing as the first pattern. A pattern was four equally spaced wells seven mm in diameter with no center well. A fourth well pattern consisted of four wells seven mm in diameter and seven mm apart in a linear fashion. Three wells of the same size were placed on either side of the four wells, one between each two center wells. All wells held approximately 0.2 ml. During formation of precipitin zones, the diffusion plates were kept at room temperatures in a moisture chamber. Zones were most easily observed with horizontal illumination against a black background.

Electronmicroscopy

A) Electronmicroscopy of BPMV

A partially purified preparation of BPMV was prepared for observation with the electron microscope by a modification of the method of Semancik and Bancroft (48), who examined the fractionated components of BPMV. The fractionated components were dialyzed against 0.01 M ammonium acetate. However, for this study, the partially purified virus preparation was dialyzed against 0.01 M pH 7.0 phosphate buffer. The virus preparation was then mixed with 2 percent uranyl acetate in a ratio of one part virus to two parts stain. After a

three to six hour staining period, a drop of the virus-stain mixture was put on a carbon-coated grid. The excess mixture was drawn off by touching to filter paper, and rinsed by dipping in distilled water 5-10 times. The grids were viewed on a Hitachi Model HU-11A electron microscope.

B) Electronmicroscopy of CPMV

A partially purified preparation of CPMV was dialyzed in the manner described for BPMV and negatively stained according to the method of Shepherd (51). A small portion of the virus was mixed with an equal volume of 2 percent phosphotungstate in 25 percent ethanol. The mixture was put on carbon-coated grids as previously described. The material was examined on the Hitachi Model HU-11A electron microscope.

EXPERIMENTAL RESULTS

Symptomatology and Host Range

BPMV produced local lesions on some bean varieties and systemic mottle on others. The local lesions (Plate 2) on Pinto and Great Northern (Plate 1) were somewhat circular and appeared two to four days after inoculation. They were light brown, appeared to be subepidermal and were 1-2.5 mm in diameter with an average of two mm. The size of the lesions depended on the age of the leaf inoculated and the number of lesions per unit area. Fewer and smaller local lesions were produced on the older leaves. On some hosts very small "pinpoint" local lesions were produced on leaves inoculated with BPMV. Soybean (G. max), beggarweed (Desmodium spp.), scarlet runner (Phaseolus coccineus), sicklepod (Cassia obtusifolia) and various species of the genus Phaseolus were inoculated with BPMV. A systemic mottle was produced in the new growth terminal to the inoculated leaves. On soybean, vein-clearing and mild mottle (Plate 6) on the first trifoliate were the first noticeable symptoms which faded within a week or two depending on the temperature at which the plants were grown. Later a severe mottle was evident on the succeeding trifoliates, followed by blistering, puckering and chlorosis. BPMV infected plants were slightly to severely stunted depending on the variety. However, in no case was the virus observed to cause necrosis. The results of the BPMV host range are given in Table 1.

CPMV produced necrotic local lesions on P. vulgaris var. Pinto and Great Northern (Plates 3, 4) and systemic symptoms on Vigna spp.

and G. max (Table 1). On soybeans CPMV produced severe mosaic and necrotic spotting (Plate 7). The leaflets of the first trifoliate had shortened petioles and were severely stunted as was the entire plant. On Early Ramshorn cowpeas (V. sinensis) the first symptoms were faint local lesions which were visible on the inoculated leaves (Plate 5) and which later became darkened and necrotic. Vein-clearing was evident on the first trifoliate, followed by a mottling. The first and subsequent trifoliates developed severe mosaic which consisted of alternate irregular patches of light and dark green tissue (Plate 9). The edges of the leaflets were folded down in a convex cup-shape very similar to that described by McLean (34).

Environmental Effects

When plants that had been inoculated with BPMV and CPMV, respectively, were placed in growth chambers at 20, 25, 30, 35 and 40 C, the time of appearance of and intensity of symptoms varied depending at what temperature they were incubated. Daily observations were made following the appearance of the first trifoliate for a period of 10 days.

Plants in the 20 C chamber grew slowly. Trifoliate leaves of the controls were usually fully expanded within two days after the lateral leaves had reached full size. At 20 C, however, the first trifoliate leaves of infected plants had only begun to emerge and were fully expanded five days later. Observations were then made daily. On the BPMV infected plants vein-clearing was the first noticeable symptom followed by a severe chlorotic mottle on the first trifoliate. Subsequent trifoliates developed the mottling followed by

severe puckering and blistering of the leaflets. The CPMV inoculated plants also showed vein-clearing. This was followed by a slight mottling and formation of irregular patches of lighter green alternating with the normal green in the leaflets. Stunting of the plants was accompanied by a severe mosaic.

At 25 C vein-clearing was visible in the first trifoliates of BPMV and CPMV inoculated plants three days after inoculation. Five days following inoculation, a slight mosaic pattern was observed on the CPMV infected plants. The BPMV infected plants showed only a slight mottle at the end of the five day period. At 10 days the BPMV symptoms had begun to fade.

The plants in the 30 C growth chamber had more intense expression than those at the previous two temperatures. Three days after inoculation, vein-clearing occurred on the first trifoliates of CPMV infected plants. A mild mosaic was observed which became more severe by the sixth day. At the end of the ten day period, the severity of the symptoms had not increased on the CPMV infected plants. During the same time interval BPMV produced a vein-clearing and a mild mottle on the first trifoliates. The mottling became more severe until the sixth day when fading became evident.

At 35 C symptom expression of BPMV was retarded until six days following inoculation when a slight mottle and chlorotic spotting appeared on the first trifoliates. The severity of the mottling and chlorosis did not increase by the end of the ten day period when symptoms had almost faded. The CPMV infected plants showed vein-clearing and mild mosaic cymptoms on the first trifoliates

four days following inoculation. No further change was noted in the symptoms by the tenth day.

There was complete supression of symptoms on the plants in the 40 C growth chamber for the entire ten day period.

Transmission Studies

A) Insect Transmission

The results of insect transmission tests showed that the bean leaf beetle (C. trifurcata) was a vector for BPMV and CPMV (Table 2). BPMV was transmitted in six of 20 attempts or with 30 percent efficiency by the bean leaf beetles. CPMV was transmitted in two of 20 attempts or with 10 percent efficiency by the bean leaf beetle. Attempts to transmit BPMV and CPMV with Myzus persicae (green peach aphid) were unsuccessful.

B) Seed Transmission

Of 500 seeds from BPMV and CPMV infected soybeans, there was a greater amount of germination of the seeds from the CPMV infected plants than seeds from those plants infected with BPMV. Clay and Early Ramshorn cowpeas for CPMV infected plants also showed good germination. However, no seed transmission of either the BPMV or the CPMV soybeans or cowpeas was observed. Sap from plants suspected of having a seed-borne virus failed to show local lesions on their respective indicator plants.

Physical Properties

The maximum dilution of BPMV and CPMV from infective leaf extract was 10^{-5} (Table 3). Thermal inactivation for both BPMV and

CPMV was between 60 C and 70 C (Table 4). Longevity in vitro at 22 C for infection with BPMV and CPMV was four days but none at six days.

Purification

Purification of BPMV according to the method of Bancroft (6) yielded smaller amounts of virus than that reported by him. The highest yield for BPMV obtained from approximately 700 grams of soybean tissue was 0.64 mg virus/ml, $A\frac{280}{260} = 1.49$, $A\frac{max}{min} = 1.22$. Figure 1 shows the absorption spectrum for BPMV which is typical for nucleoproteins. The absorption maxima and minima were near 260 and 240 mu, respectively.

CPMV yielded 1.7 mg virus/ml for 500 grams of cowpea tissue. Figure 2 shows the typical nucleoprotein absorption spectrum for CPMV after four cycles of differential centrifugation. The absorption maxima and minima were 250 and 265, respectively, $A\frac{260}{280} = 1.11$, and $A\frac{max}{min} = 1.40$.

Serology

Plates 10, 11, 12 show the results of the agar-diffusion tests. When the BPMV antiserum was tested against its homologous antigen and against the North Carolina isolate antiserum diffusion zones were formed (Plate 10). Plate 11 shows the agar gel-diffusion test comparing the four BPMV to Louisiana and North Carolina isolate antigen. The North Carolina antigen reacted with both its homologous antiserum and the antiserum to the Louisiana isolate. The Arkansas and the Indiana isolates, however, (Plate 11, A, D) produced no reaction to the antigen.

Tests comparing the CPMV antigen to its homologous antiserum and to the antiserum from the California isolate of CPMV failed to show a reaction. This was probably due to the low titer in the CPMV preparation. However, when clarified, infective sap was used, a precipitation reaction occurred between the infective sap and the two antisera (Louisiana and California isolates) as shown in Plate 12. In all agar gel-diffusion tests used in this study, normal serum to each antiserum was used as a control.

Varietal Response

In both the 1969 and 1970 field tests with six varieties of soybeans infected with BPMV, the yields were not significantly less than those from the controls (Table 5). Analysis of variance revealed no significant differences between BPMV infected and control plants, and no significant interaction between varieties and virus.

However, there were significant differences between the yields of the CPMV inoculated varieties and the corresponding control plants at the 1 and 5 percent levels of L.S.D. Hill was the only variety in which the differences between the average mean yields of soybeans from infected plants and controls were not significant at the 1 and 5 percent levels. Of the remaining four varieties average yields of inoculated plants were significantly lower than those of the controls (Table 6).

In greenhouse tests BPMV reduced the yields of Lee, Hill, Dare, and Semmes which were significantly lower than the yields of similar healthy plants. The two varieties, Bragg and Davis were apparently

resistant to BPMV since the yields of neither variety were significantly lower than the yields of the healthy controls (Table 7).

In the greenhouse the yields of all varieties were significantly reduced to the 1 percent level when they were inoculated with CPMV. CPMV produced a profoundly severe effect on these varieties as shown in Table 8.

Table 1. Plants inoculated with BPMV and CPMV, respectively, and their reactions to them.

Plant Species	Type of Reaction[2] BPMV	CPMV
Albizia julibrissin Durazzin (Mimosa)	-	-
Apios americana Medicus	-	-
Arachis hypogaea L. (Peanut)	-	-
Capsicum annuum L. (Bell Pepper, Yolo Wonder)	-	-
Cassia obtusifolia L. (Sicklepod)	S	-
Cassia marilandica (Wild Senna)	-	-
Centrosema americana Bentham (Butterfly Pea)	S	-
Cercis canadensis L. (Redbud)	-	-
Chenopodium amaranticolor Coste & Reyn. (Pigweed)	-	LL
Chenopodium quinoa (Pigweed)	-	LL
Clitoria mariana L. (Butterfly Pea)	-	-
Crotalaria spp. (Rattlebox)	-	-
Cucumis melo L. (Muskmelon)	-	-
Cucumis sativus L. (Cumcumber, NPC)	-	-
Cucurbita pepo L. (Little Sugar Pumpkin)	-	-
C. pepo L. var. melopepo Alef. (Squash)	-	-
Datura stramonium L. (Jimson weed)	-	-
Desmodium canadense L. (Beggarweed)[1]	S	-
D. laevigatum L. (Beggarweed)[1]	S	-
D. paniculatum L. (Beggarweed)	S	-

[1] Host not previously reported.

[2] S, systemic symptoms, LL, local lesions

Table 1. (Continued)

Plant Species	Type of Reaction[2] BPMV	CPMV
Gomphrena globosa L. (Globe Amaranth)	-	-
Gleditsia triacanthos L. (Honeylocust)	-	-
Glycine max (L.) Merr. (Soybean)		
Var. Bragg	S	S
var. Dare	S	S
var. Davis	S	S
var. Hill	S	S
var. Lee	S	S
var. Semmes	S	S
Helianthos annus L. (Sunflower)	-	-
Lupine mutabilis L. (Sweet Lupine)	-	-
Lathyrus odoratus L. (Sweet Pea)	-	-
Lycopersicon esculentum Mill. (Tomato, var. Creole)	-	-
Medicago sativum L. (Alfalfa)	S	-
Nicotiana glutinosa L.	-	-
N. tabacum L.		
var. Havana 425	-	-
var. N.C. 95	-	-
Phaseolus vulgaris L.		
var. Bountiful Bush	LL	LL
var. Common Bush Bean	-	-

[2] S, systemic symptoms, LL, local lesions

Table 1. (Continued)

Plant Species	Type of Reaction[2] BPMV	CPMV
Phaseolus vulgaris L.		
var. Contender Bush Bean	LL	-
var. Great Northern	LL	LL
var. Green-seeded Henderson	-	LL
var. Harvester Bush Bean	LL	-
var. Jackson Wonder	-	LL
var. Kentucky Wonder Wax Pole Bean	LL	LL
var. Pinto	LL	LL
Phaseolus coccineus L. (Scarlet Runner)	S	-
var. Albonanus (White Bean)	-	-
Phaseolus lunatus L.		
var. Henderson Bush Lima	-	LL
var. Willow Leaf Pole Lina	-	LL
var. Sieva Bean	-	LL
Physalis spp. L. (Ground Cherry)	-	-
Pisum sativum L.		
var. Arvense (Austrian Winter Pea)	-	-
var. Laitonian English Pea	-	-
Pueraria thunbergiana (Siebold & Zucc.) Bentham (Kudzu)[1]	-	-
Sesbania exalta (Raf.) Cory (Teaweed)	-	-

[2] S, systemic symptoms, LL, local lesions

Table 1. (Continued)

Plant Species	Type of Reaction[2] BPMV	CPMV
Sorghum vulgare Pers. (Sorghum)	-	-
Trifolium hydridum L. (Alsike Clover)	-	-
Trifolium incarnatum L. (Crimson Clover)	-	-
Trifolium pratense L. (Red Clover)	-	-
Trifolium repens L. (La. White Clover)	-	-
Vicia angustifolia Reichard (Narrow Leaf Vetch)	-	-
Vicia faba L. (Broad Bean)	-	-
Vicia sativa L. (Common Vetch)	-	-
Vicia villose Roth. (Hairy Vetch)	-	-
Vigna sesquipedalis L. (Yard Long Bean)	-	LL
Vigna sinensis Torner var. Early Ramshorn (Cowpea)	-	S,LL
var. Clay	-	S,LL
var. Monarch	-	S
var. Silver Skin Crowder	-	-

[2] S, systemic symptoms, LL, local lesions

Table 2. Insect transmission of BPMV and CPMV, respectively, in the greenhouse.[1]

Vector	No. insects tested		No. plants infected		Percentage infection	
	BPMV	CPMV	BPMV	CPMV	BPMV	CPMV
C. trifurcata	20	20	6	2	30	10
M. persicae	20	20	0	0	0	0

[1] One insect per plant.

Table 3. The dilution end-point of BPMV and CPMV from infected leaf extracts.

Dilution	Number of plants inoculated		Number of plants infected	
	BPMV	CPMV	BPMV	CPMV
Control	12	12	12	12
1:1	12	12	12	12
1:10	12	12	12	12
1:100	12	12	12	12
1:1,000	12	12	12	12
1:10,000	12	12	10	1
1:100,000	12	12	0	0
1:1,000,000	12	12	0	0

Table 4. The thermal inactivation of BPMV and CPMV from infected leaf extracts.

Temperature (degrees Cent. for 10 min.)	Number of plants inoculated		Number of plants infected	
	BPMV	CPMV	BPMV	CPMV
Control	12	12	12	12
60	12	12	12	12
70	0	0	0	0
75	0	0	0	0
80	0	0	0	0
90	0	0	0	0

Table 5. The average mean yields in pounds of soybeans from healthy and BPMV inoculated plants grown in the field.

Variety	Control plants		BPMV infected plants		Difference	
	1969	1970	1969	1970	1969	1970
Hill	6.48	6.00	5.10	7.00	1.38	1.00
Dare	7.92	8.00	6.92	7.20	1.00	1.80
Davis	9.70	10.00	8.50	10.30	1.20	0.30
Lee	7.78	4.80	7.60	4.10	0.18	0.60
Bragg	8.55	5.90	8.48	5.20	0.07	0.70
Semmes	7.00	6.90	6.25	6.40	0.75	0.50

Table 6. The average mean yields in pounds of soybeans from healthy and CPMV inoculated plants grown in the field.

Variety	Control Plants	CPMV Infected Plants	Difference
Hill	5.8	4.4	1.4
Davis	11.6	6.1	5.5
Lee	6.9	2.8	4.1
Bragg	6.2	3.0	3.2
Semmes	6.0	2.5	3.5

L.S.D. = 5% = 2.3
1% = 3.2

Table 7. The average mean yields in grams of soybeans from healthy and BPMV infected plants grown in the greenhouse.

Variety	Control Plants	BPMV Infected Plants	Difference
Hill	77.02	54.52	22.50
Dare	71.22	40.50	20.72
Davis	44.32	47.95	3.63
Lee	93.08	65.62	27.46
Bragg	59.78	66.27	6.49
Semmes	59.0	45.77	13.23

L.S.D. = 5% = 19.7
1% - 26.56

Table 8. The average mean yields in grams of soybeans from healthy and CPMV infected plants grown in the greenhouse.

Variety	Control Plants	CPMV Infected Plants	Difference
Hill	70.12	16.77	53.35
Dare	80.65	10.75	69.90
Davis	71.32	18.02	53.50
Lee	69.45	14.05	55.40
Bragg	78.57	14.82	63.75
Semmes	78.17	12.60	65.57

L.S.D. = 5% - 16.02
1% = 21.58

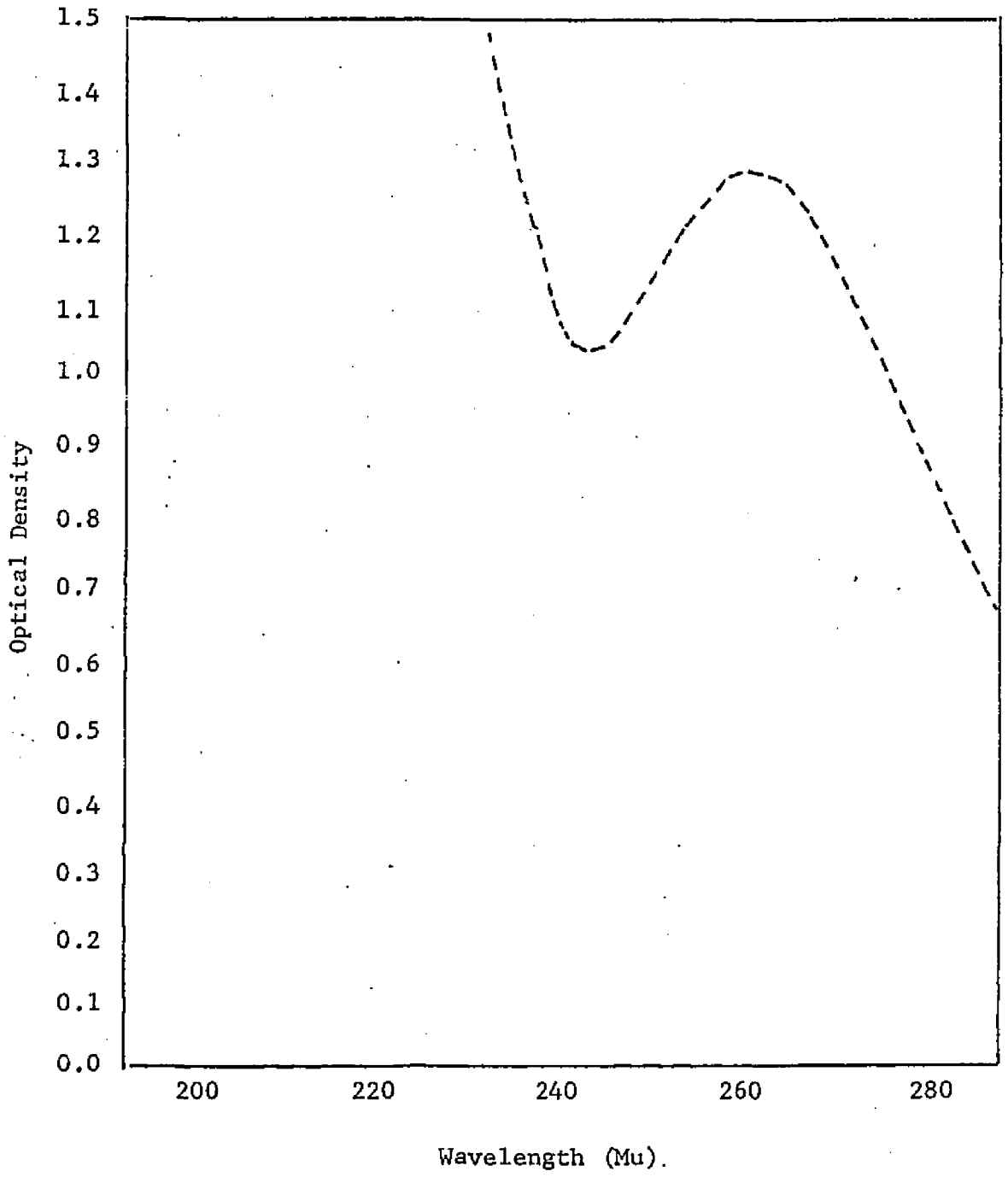

Figure 1. Absorption spectrum of a purified preparation of the Louisiana isolate of BPMV. The curve is a tracing from a Perkin Elmer Spectrophotometer.

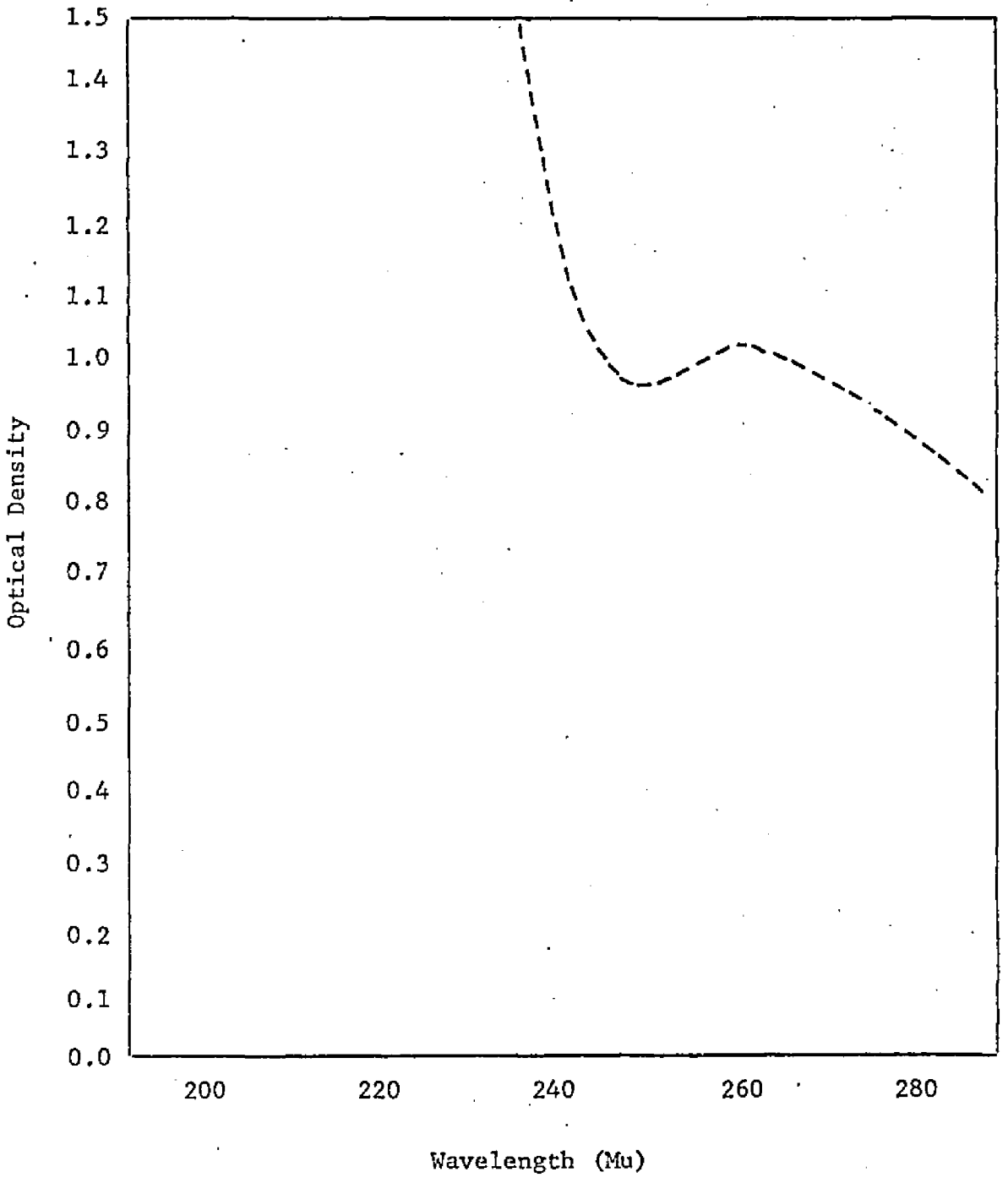

Figure 2. Absorption spectrum of a purified preparation of the Louisiana isolate of CPMV. The curve is a tracing from a Perkin Elmer Spectrophotometer.

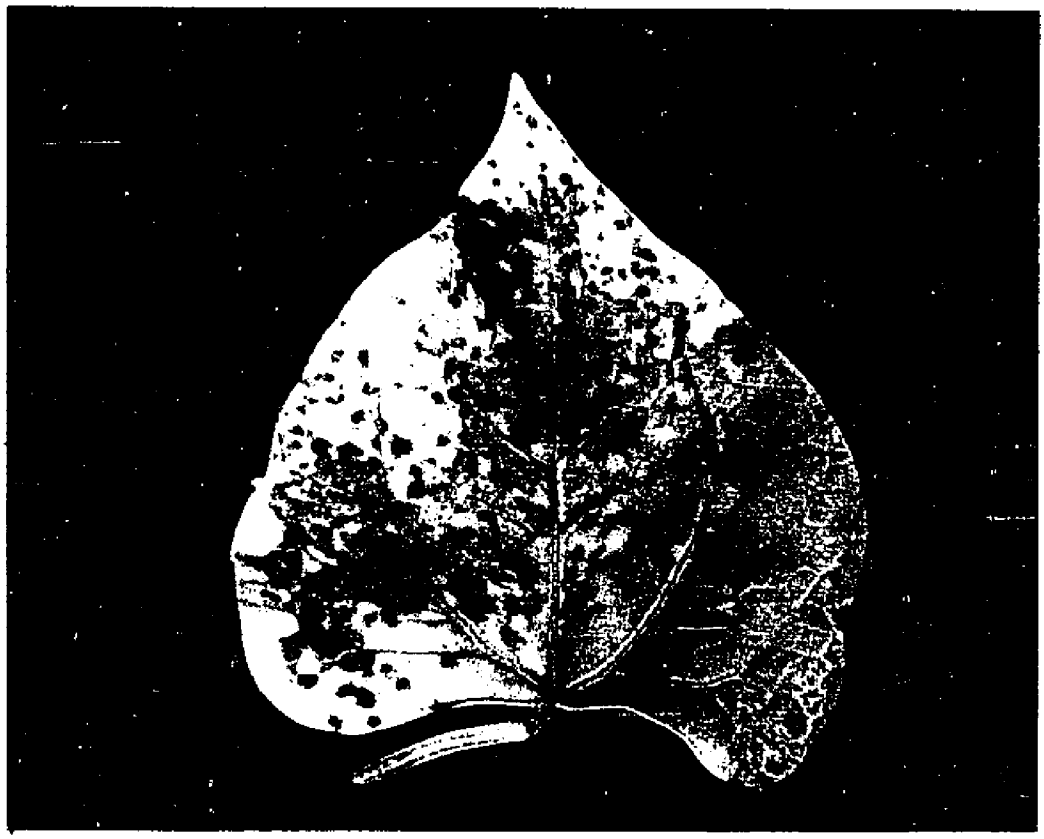

Plate 1. Local lesions produced by the Louisiana isolate of BPMV on Great Northern.

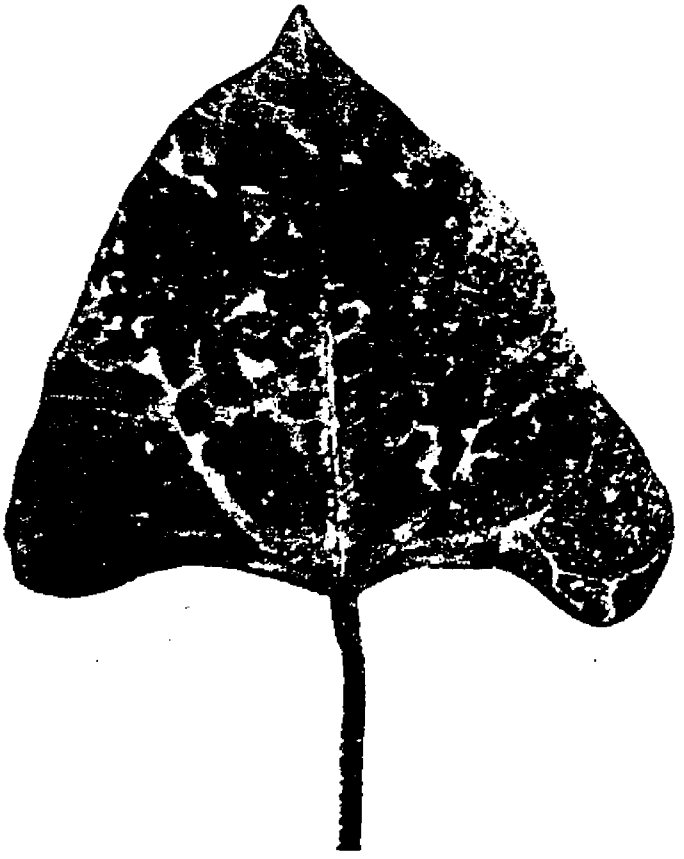

Plate 2. Local lesions produced by the Louisiana isolate of BPMV on Pinto.

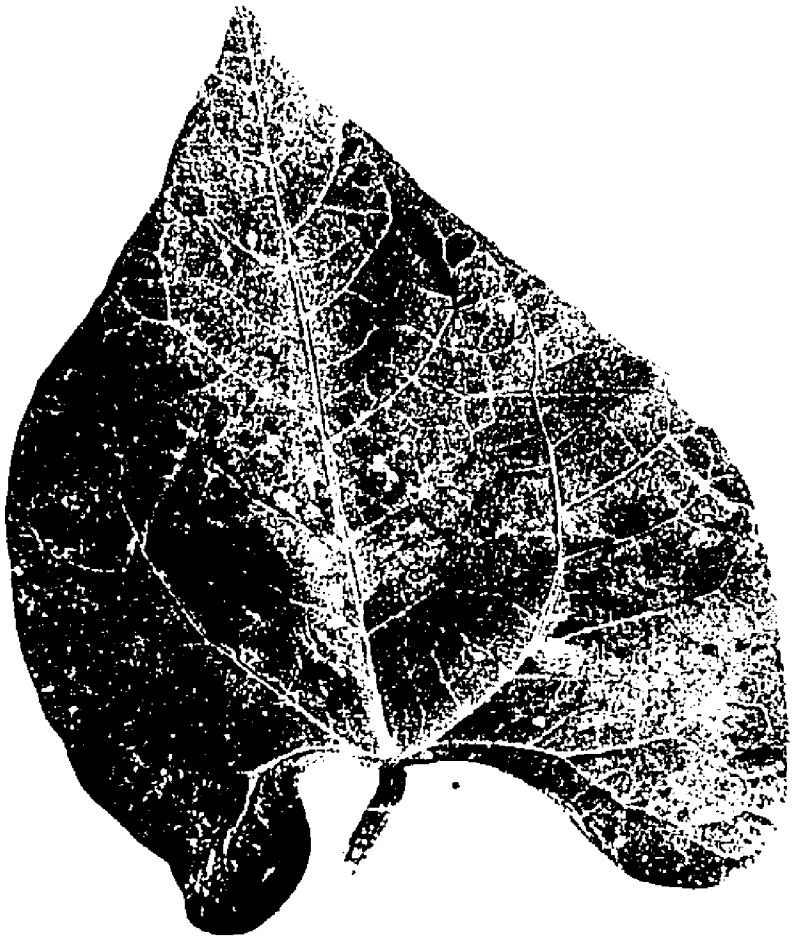

Plate 3. Local lesions produced by the Louisiana isolate of CPMV on Great Northern.

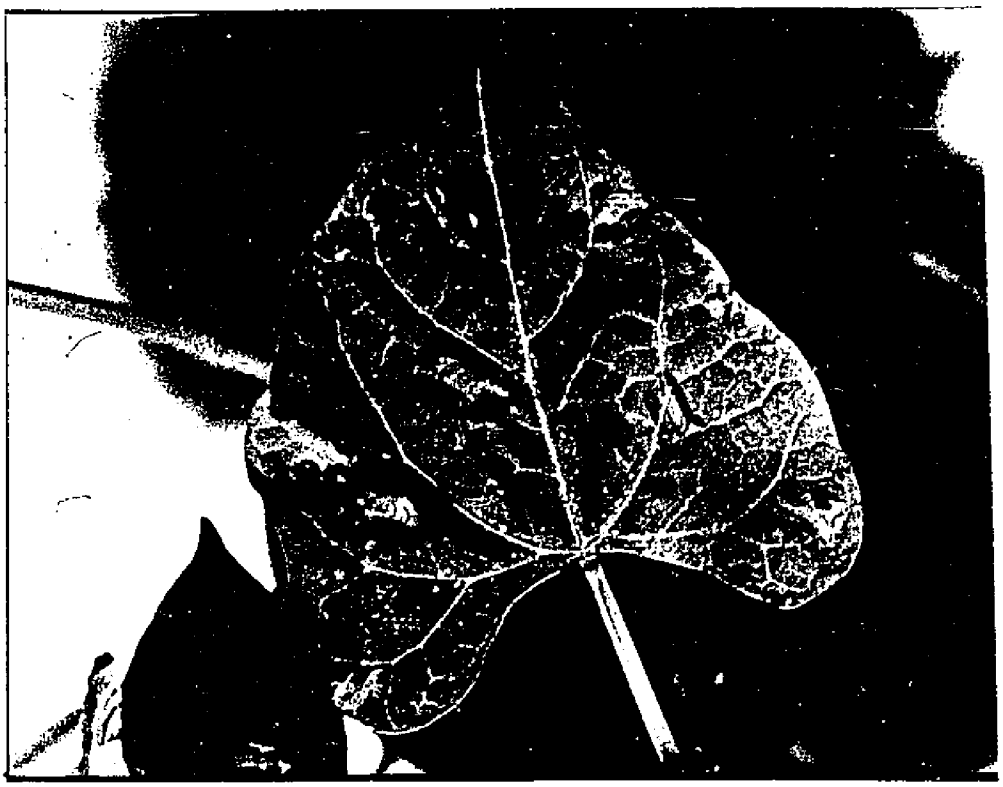

Plate 4. Local lesions produced by the Louisiana isolate of CPMV on Pinto.

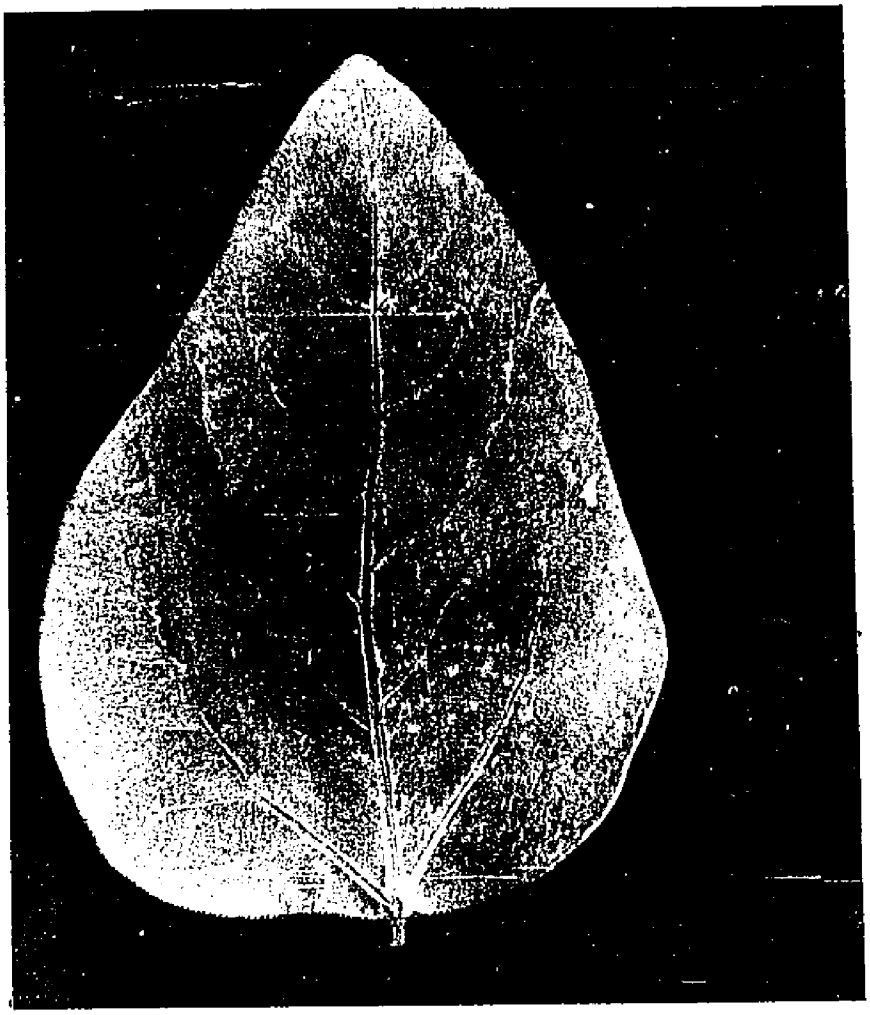

Plate 5. Local lesions produced by the Louisiana isolate of CPMV on cowpea.

Plate 6. Systemic mottle on Dare soybean leaves caused by the Louisiana isolate of BPMV.

Plate 7. Systemic symptoms on Dare soybean leaves caused by the Louisiana isolate of CPMV.

Plate 8. Healthy Dare soybean.

Plate 9. Systemic symptoms on Early Ramshorn cowpea leaves caused by the Louisiana isolate of CPMV.

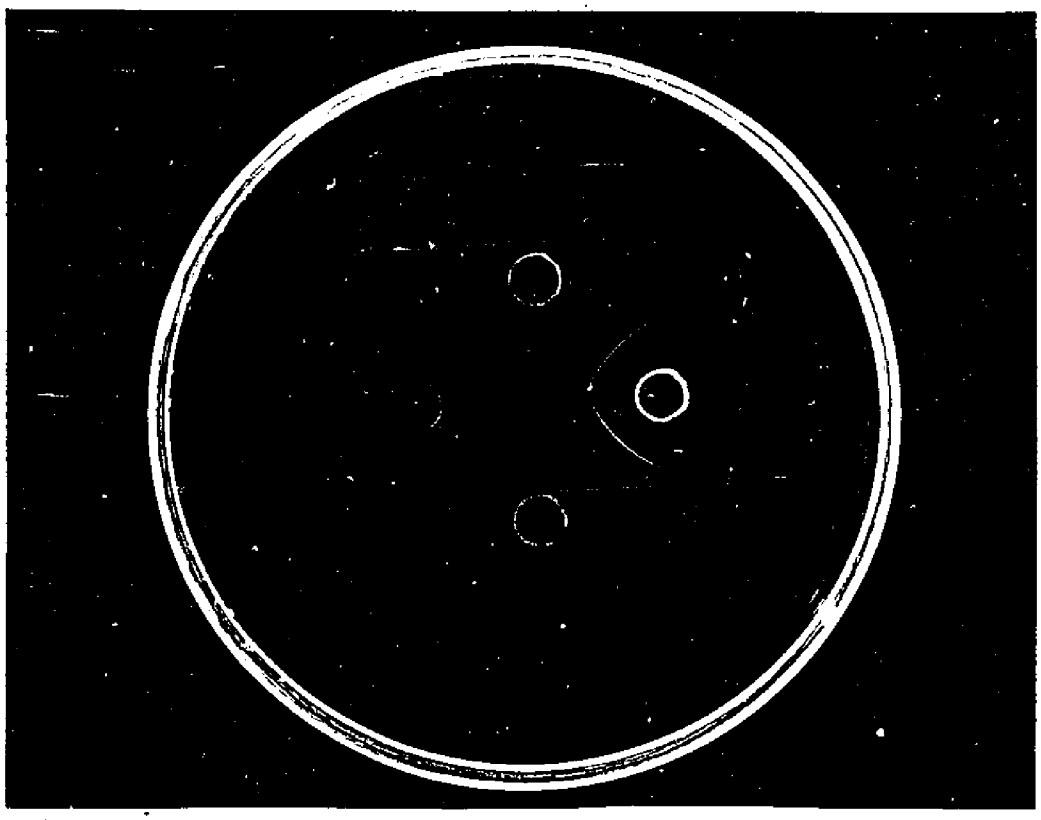

Plate 10. Ouchterlony gel-diffusion test comparing the Louisiana isolate of BPMV antigen (B) to its homologous antiserum (A) and to the North Carolina isolate antiserum (C). (D) contains antigen to the Louisiana BPMV isolate.

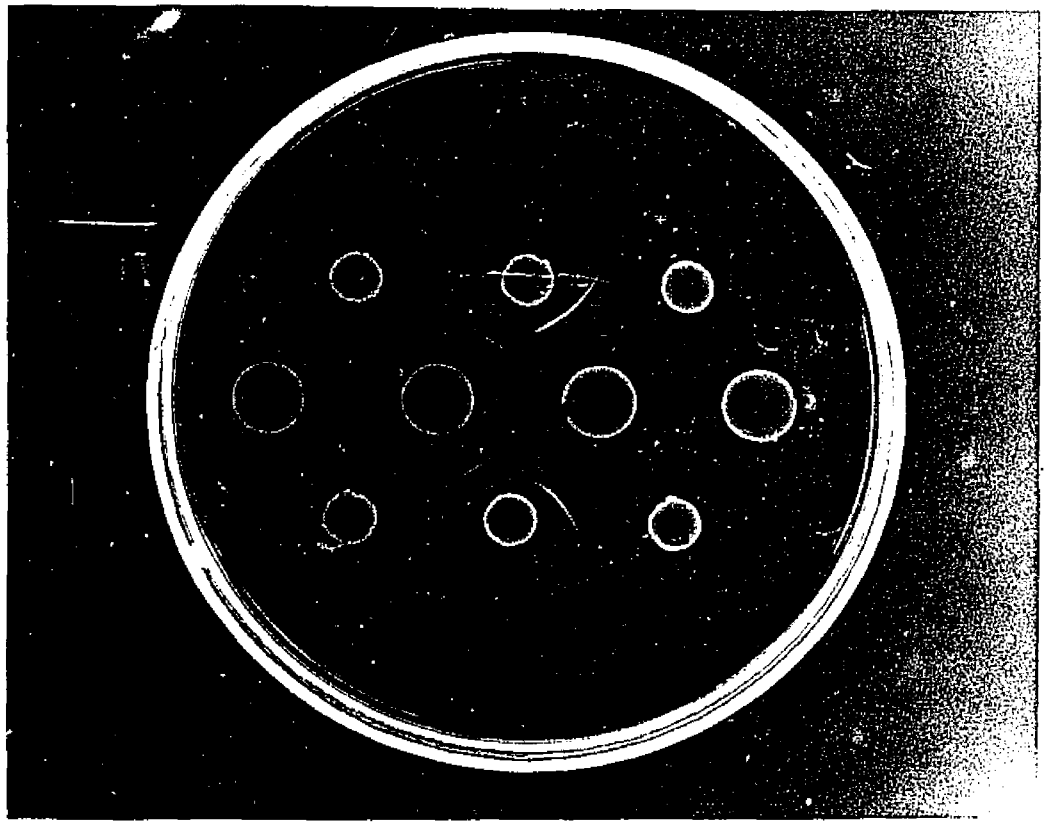

Plate 11. Ouchterlony gel-diffusion test comparing four BPMV antisera: Indiana isolate (A), Louisiana isolate (B), North Carolina isolate (C), and Arkansas isolate (D) with homologous antigen to the Louisiana isolate (E, H, J, G) and with the North Carolina BPMV antigen (I, F).

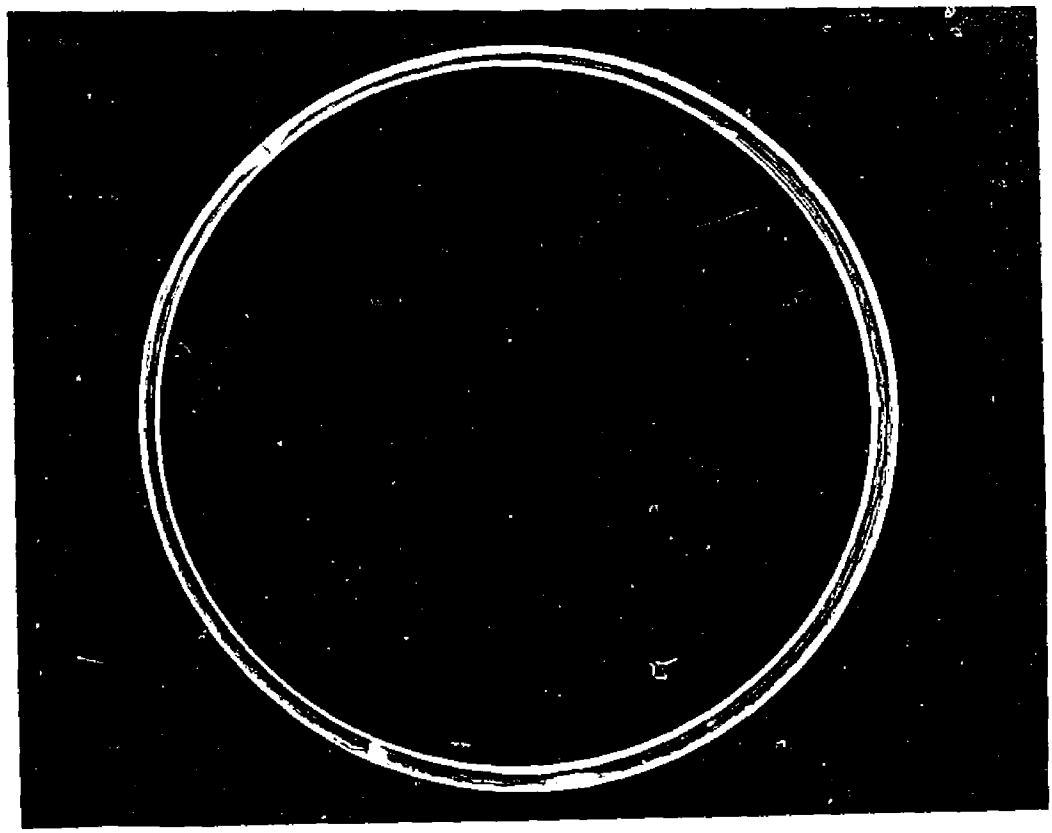

Plate 12. Ouchterlony gel-diffusion test comparing CPMV antigen (infected sap) (C) with its homologous antiserum (D) and with antiserum from the California isolate of CPMV (B). Well (A) contains sap from healthy Dare soybean.

DISCUSSION

There are many problems associated with the identification of viruses found in legumes in the field (10). First, the probable virus isolates must be separated from a possible "virus complex" because multiple virus infection in legumes is not uncommon. A detailed study must then be made of each isolate. Among the properties of the virus to be investigated are symptomatology, host range, influence of environment, transmission methods, physical properties, serology, and electronmicroscopy. Virus identification based on host reaction alone is of limited usefulness, since symptoms often depend, among other factors, on the species or variety used and on environmental conditions (1, 6, 7). Consequently, the so-called "clinical diagnosis" becomes unreliable (10). Therefore, greater emphasis must be placed on the study of the intrinsic characters of the viruses by physicochemical tests and especially electronmicroscopy and serology. Thus far, the study of these properties has been limited to viruses which are sap transmissable (10).

Zaumeyer and Thomas (68) described BPMV in 1948 as a new virus infecting beans (P. vulgaris). On some plants, the virus produced circular local lesions, while on others, a chlorotic mottle was produced. Results of the host range study with the BPMV isolate used in this study demonstrated that local lesions were produced in six varieties of P. vulgaris and systemic symptoms were produced on Cassia obtusifolia L., Centrosema americana Bentham, Desmodium canadense L., Desmodium laevigatum L., Desmodium paniculatum L. and

G. max. The symptoms agreed closely with those reported by Zaumeyer and Thomas (68) on P. vulgaris var. Longreen and Tendergreen. Skotland (52) also reported on a host range study with BPMV. The results (Table 1) also agree with the results of Skotland (52) with the exception that no symptoms were produced on cowpea (V. sinensis) and crimson clover (T. incarnatum) with the Louisiana isolate. The systemic symptoms produced on Desmodium spp. indicated a relation of the virus to the BPMV isolate described by Walters (61) and Moore et al. (35). BPMV produced a systemic mottling in all varieties of soybeans tested. A similar reaction was reported by Moore, et al. (35), Ross (41, 42, 43, 44, 45), Walters (61), and Walters and Barnett (62). These results indicated a close similarity between these BPMV isolates and the Louisiana isolate.

The symptoms produced by the Louisiana CPMV isolate were very similar to the symptoms of CPMV described by Elliot (21) and by McLean (34). Of the 67 plants inoculated in the host range study, (Table 1) 18 showed symptoms when inoculated with CPMV. Local lesions were produced in C. amaranticolor, C. quinoa, Phaseolus spp., V. sesquipedalis, and V. sinensis. Systemic symptoms were produced on G. max and V. sinensis. McLean (34) also reported that V. sinensis was susceptible to CPMV. The host range for the CPMV (Louisiana isolate) differed from the host range given by Shepherd and Fulton (50). They reported a rather limited host range for a CPMV that was closely related serologically to southern bean mosaic virus (SBMV). The only legumes showing a reaction to their CPMV isolate was P. sativum, G. max, and V. sesquipedalis. The latter two were hosts for the Louisiana isolate of CPMV. Shepherd (51), however, described

another CPMV isolate with a broader leguminous host range in addition to several non-leguminous hosts. This latter isolate agreed more closely with those listed as hosts of the Louisiana isolates shown in Table 1. The host range described for the Trinidad strain of CPMV (13) included some of the hosts of the Louisiana CPMV isolate listed in Table 1. It was interesting to note that the California CPMV isolate (50) that is similar to SBMV, the Trinidad isolate (13), and the Louisiana isolate all produced symptoms on P. vulgaris and C. amaranticolor.

The results of the environmental effects on BPMV and CPMV indicated that temperature plays a major role in symptom expression. Dare soybean plants were inoculated with BPMV and CPMV, respectively, and placed in growth chambers at 20, 25, 30, 35 and 40 C. The two extreme temperatures had the most pronounced effect on symptom expression of both viruses. Symptom expression was most intense when inoculated plants were incubated at 20 C. Symptoms at this temperature were characteristic of those described by other workers. It is of interest to note that when the plants incubated at 20 C were removed from the growth chamber and placed in an air-conditioned greenhouse (26 C), the symptoms faded. However, within a week the symptoms of both viruses were manifested as before. This phenomenon is similar to that described by several workers (6, 44, 68). No symptoms were observed on inoculated plants incubated at 40 C. This suggested a possible inactivation of the viruses. Symptom expression of inoculated plants at 25, 30 and 35 C was not changed markedly except those maintained at 30 C produced slightly more severe symptoms. Thus, it appeared from these observations that symptom expression of BPMV

and CPMV, respectively, was influenced by temperature. Temperatures of 20-30 C appeared to be optimum for expression of symptoms, 35 C supressed them and none were observed at 40 c.

In the 1969 and 1970 field tests, no significant differences in yields of beans were obtained between the inoculated BPMV plants and the controls. These results contrast sharply with Ross's (41, 44) results on yield response of singly and doubly infected plants inoculated with SMV and BPMV. He obtained yield reductions on Lee (80 percent) and Hill infected with BPMV. Table 5 indicates no significant reduction in yields of either Lee or Hill. It can be pointed out here that the difference in the mean yield between the infected and the control plants of Hill is in fact, a negative number. These results could possibly be explained by the fact that 100 percent infection of all plants in the plot was not obtained. Failure to get 100 percent infection was probably due to an excessive number of plants in the test plots. Greenhouse yield tests on varietal response (Table 7), however, showed that there was a highly significant difference between the BPMV infected plants and the control plants. It is significant to note here that Hill and Lee had the greatest reductions in yields. This correlates closely with the results obtained by Ross (41, 44). The greater reduction in yields obtained in greenhouse tests compared with field tests was due to the percent of infection. Greenhouse plants were 100 percent infected, whereas it is believed that the percent infection in the field was extremely low.

There was some degree of significance between CPMV infected plants and healthy plants grown in the field. The data in Table 6

indicates a significant difference at the one and five percent levels of probability. There is little reason to believe here that a higher percentage of infection was obtained for CPMV than for BPMV. CPMV was a much more severe disease in soybeans than BPMV. It caused severe stunting that resulted in smaller yields than BPMV infected plants. The greenhouse tests with CPMV infected plants (Table 8) indicated similar results as the BPMV greenhouse tests. Reasons given for the greater reductions in yields in the greenhouse tests as compared with field tests with CPMV are the same as stated previously when BPMV was the virus discussed.

BPMV was shown (Table 2) to be transmitted by C. trifurcata (bean leaf beetle). However, transmission was only 30 percent as compared to Walters (63) who obtained 53 percent transmission of BPMV by C. trifurcata, and Ross (42) who reported 67 percent transmission of BPMV by C. trifurcata. Horn et al. (29) also reported 15 percent transmission of BPMV with C. trifurcata and whose results more nearly approximated those in Table 2. The transmission of BPMV reported by Horn et al. (29) for other Chrysomelid beetles was 4 to 7 percent. Many factors could account for these differences in amount of transmissability. Among these are the source plant, temperature, humidity, light variations, acquisition of the virus by the insect and the length of time that the insect remains viruliferous after acquisition of the virus. Not to be overlooked too, is the titer of the virus in the source plant at the time of feeding. M. persicae was not a vector for BPMV. Similar results have been reported by other workers (42, 64, 65).

Table 2 shows only 10 percent transmission of CPMV with C. trifucata. Nevertheless, these results establish that the virus is related to other CPMV strains that have been reported as being transmitted by C. trifucata (2, 53, 61). The reasons given for the low percentage of transmission of CPMV may be due to the same factors that caused the low transmission level of BPMV. Attempts to transmit CPMV with M. persicae were unsuccessful. However, Yu (67) obtained an extremely high percentage of infection with Aphis rumicis L. (black aphid), Macrosiphum pisi Kalt. (pea aphid), and Aphis gossypii Glov. (cotton aphid). In some cases Yu (66) noted 100 percent infection with aphids.

No seed transmission of either BPMV or CPMV was observed. These results agree with those of Skotland (52) who also reported no seed transmission for BPMV, and with those of Perez and Monllor (38) who reported no seed transmission for seed from CPMV infected Early Ramshorn cowpeas (V. sinensis). However, seed transmission of CPMV was reported by a number of workers (19, 26, 34, 50, 53, 66). Although CPMV showed a relationship to the California CPMV isolate, which was reported to be seed-borne by Shepherd and Fulton (50), there was no seed transmission in either soybeans or cowpeas.

The dilution end-point (DEP) of BPMV was found to occur at 1;10,000 (Table 3). Zaumeyer and Thomas (68) reported a similar result for the DEP of BPMV. The DEP for CPMV was also found to be 1:10,000, although infection at this dilution was limited to one plant (Table 3). The work of Shepherd and Fulton (50), Shepherd (51), and Chang (13) on the DEP of CPMV support this value. McLean (34),

however, indicated that the DEP of CPMV occurred at 1:1,500, and Yu (67) reported 1:2,500 as the DEP of CPMV.

Thermal inactivation studies with BPMV showed that infection occurred at 60 C, but none at 70 C (Table 4). This value was slightly lower than that given by Zaumeyer and Thomas (68) which was between 70 and 80 C for inactivation of BPMV. Matthews (33) lists the thermal inactivation point (TIP) for BPMV between 60 and 85 C. These values, however, represent a group of viruses and should not be interpreted as the true value for any one virus in the group. The TIP for CPMV was also between 60 and 70 C (Table 4). These figures agree with those of Chant (13) and Shepherd (51) who reported inactivation of CPMV at 70 C. These figures do not agree with those of other investigators. McLean obtained infectivity with CPMV extract up to 72 C. Yu (67) reported the TIP for CPMV at 64 C. This figure is not consistent with the results of the other workers reported here and with Shepherd and Fulton (50) who got inactivation of CPMV extract at 90 C. These two extremes suggest that the great variation of inactivation temperatures may be due to the various strains of CPMV.

Longevity in vitro (LIV) values for BPMV and CPMV, respectively, were identical. Infection was obtained at four days, but none at six days. Zaumeyer and Thomas (68) obtained a much higher figure at 62 days. A much closer figure to the one reported for this study was obtained by McLean (34) who reported inactivation between 48 and 72 hours, and by Yu (67) who observed infection at 72 hours but none at 96 hours. Other values reported for LIV of CPMV ranged from 7 days to 16 days (13, 51, 52).

The purification of BPMV was accomplished by the method described by Bancroft (6). However, considerably smaller yields were obtained than those reported by him. Loss of virus during purification is frequently attributed to variation in techniques employed by the particular worker. It is doubtful that the low virus yield was due to a low titer since local lesion assays at the time of harvest indicated a sufficiently high titer for purification. The yield of virus for CPMV was also lower than that reported by Van Kammen (58). As with BPMV, procedural variations may have led to the reduced yield since the tissue used was of sufficient virus titer as indicated by local lesion assay.

Plates 10, 11 and 12 show the results of the agar gel-diffusion test with both viruses. The reaction of the antiserum to the Louisiana BPMV isolate with the North Carolina isolate (Plate 10) indicates that these two viruses are closely related, possibly strains of the same virus. Plate 11 shows the results of the agar gel-diffusion test in which four BPMV antisera were compared with antigens of the Louisiana BPMV isolate and North Carolina isolates, respectively. Antigens for the other two isolates were not available. This reaction shows the relationship of the Louisiana and the North Carolina BPMV isolates. No diffusion reactions occurred with either the Indiana or the Arkansas isolates. The probable reason for this is the extremely low yield of virus obtained for the antigen of the Louisiana isolate.

Plate 12 shows the results of the agar gel-diffusion test in which the CPMV antigen was compared with its homologous antiserum and to the California isolate of CPMV. The test was accomplished by using clarified and concentrated CPMV infective sap as the antigen.

Preliminary tests with the purified antigen indicated that insufficient amounts of the virus were present in the antigen to cause a reaction with the antiserum. These results establish a definite relationship between the CPMV isolate used in this study and the California CPMV isolate.

Stained and shadowed grids of both virus isolates were examined with the electron microscope. Due to the low concentration of both viruses, considerable difficulty was experienced in locating an aggregate of virus particles suitable for viewing. Single particles, and in some cases groups of two to four particles were observed for both BPMV and CPMV. It was definitely established, however, that both viruses consisted of spherical particles. Observations were of insufficient clarity for photographs.

SUMMARY

1. BPMV and CPMV were found in soybeans and in cowpeas, respectively, in Louisiana. Both viruses were identified by insect transmission, physical properties, serology, host range and symptom expression.
2. BPMV produced a mottling on the leaves of soybean, while CPMV caused a severe mosaic and stunting of soybean plants. Both viruses had a wide luguminous host range, and BPMV overwinters in Desmodium paniculatum L. The optimum temperatures for symptom expression were between 20-30 C.
3. The physical properties of BPMV and CPMV were very similar. Both lost infectivity at a dilution of 10^{-5}, were inactivated between 60-70 C, and had a longevity in vitro between four and six days.
4. The North Carolina isolate of BPMV was related to the Louisiana isolate by the Ouchterlony gel-diffusion test. Diffusion zones formed by the reaction of the CPMV antigen (Louisiana isolate) with the CPMV antiserum (California isolate) indicated that the two isolates were related.
5. Electronmicroscopy showed BPMV and CPMV both to be isometric particles of approximately 30 Mu in diameter.
6. Field tests revealed no significant differences in yields between the BPMV inoculated plants and the controls. Greenhouse tests, however, showed significant differences in the yields of BPMV infected Hill, Dare and Lee soybeans and the controls. No differences were found between treated and healthy Davis, Semmes and Bragg. There were significant differences between CPMV

infected plants and controls both in the field and in the greenhouse. Hill was the only variety in the field in which the yields of CPMV inoculated plants were not significantly lower than the controls. However, the yields of Hill were severely reduced in the greenhouse by the virus.

7. Both BPMV and CPMV were found to be vectored by Ceratoma trifurcata Forst. (bean leaf beetle). Myzus persicae Sulz. (green peach aphid) did not transmit either of the viruses. Neither BPMV or CPMV was found to be seed transmitted.

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VITA

Kenneth Cosmas Gremillion was born January 12, 1939 in Moreauville, Louisiana. He was graduated from Bolton High School in Alexandria, Louisiana in May, 1957. That same month he entered the United States Navy and at the termination of a thirty-six month tour of duty he was honorably discharged.

In September of 1960, he entered Louisiana State University at Alexandria. At the completion of Junior Division Studies offered there, he entered Louisiana State University in Baton Rouge, and received the Bachelor of Science degree in General Studies in June, 1965. The author was then employed by Bariod Division, National Lead Co. as a drilling fluids engineer until June, 1966 when he began graduate studies at Northeast Louisiana University. He received the Master of Science degree in Biology in May, 1968. He is presently a candidate for the degree of Doctor of Philosophy in Plant Pathology.

EXAMINATION AND THESIS REPORT

Candidate: Kenneth Cosmas Gremillion

Major Field: Plant Pathology

Title of Thesis: Bean Pod Mottle Virus and Cowpea Mosaic Virus in Louisiana Soybeans

Approved:

N. L. Horn
Major Professor and Chairman

Max Goodrich
Dean of the Graduate School

EXAMINING COMMITTEE:

S. C. Tucker

M. T. Henderson

Lt. John P. Chilton

L L Forbes

Date of Examination:

July 20, 1971